Neurochemical Research, Vol. 24, No. 2, 1999, pp. 321–329

Energetics of Functional Activation in Neural Tissues*

Louis Sokoloff[1,2]

(Accepted July 22, 1998)

Glucose utilization ($lCMR_{glc}$) increases linearly with spike frequency in neuropil but not perikarya of functionally activated neural tissues. Electrical stimulation, increased extracellular $[K^+]$ ($[K^+]_o$), or opening of Na^+ channels with veratridine stimulates $lCMR_{glc}$ in neural tissues; these increases are blocked by ouabain, an inhibitor of Na^+,K^+-ATPase. Stimulating Na^+,K^+-ATPase activity to restore ionic gradients degraded by enhanced spike activity appears to trigger these increases in $lCMR_{glc}$. Cultured neurons behave similarly. Astrocytic processes that envelop synapses in neuropil probably contribute to the increased $lCMR_{glc}$. $lCMR_{glc}$ in cultured astroglia is unaffected by elevated $[K^+]_o$ but is stimulated by increased intracellular $[Na^+]$ ($[Na^+]_i$), and this stimulation is blocked by ouabain or tetrodotoxin. L-Glutamate also stimulates $lCMR_{glc}$ in astroglia. This effect is unaffected by inhibitors of NMDA or non-NMDA receptors, blocked by ouabain, and absent in Na^+-free medium; it appears to be mediated by increased $[Na^+]_i$ due to combined uptake of Na^+ with glutamate via Na^+/glutamate co-transporters.

KEY WORDS: [^{14}C]Deoxyglucose; Na^+,K^+-ATPase; glutamate; astrocyte; sodium; potassium; glucose utilization.

INTRODUCTION

The [^{14}C]deoxyglucose ([^{14}C]DG) method (1) provided the means to measure rates of glucose utilization ($lCMR_{glc}$) simultaneously in all local regions of the nervous system. Its application in numerous studies in conscious animals and man have established that functional activation stimulates energy metabolism in neural tissues as it does in other tissues (2). The magnitude of the increases in energy metabolism bears a quantitative relationship to the degree of functional activation. For example, retinal stimulation by light flashes increases $lCMR_{glc}$ in proportion to the logarithm of the light intensity in structures of the brain that receive direct projections from the retina (Fig. 1) (2,3). Electrical stimulation of the cervical sympathetic trunk or sciatic nerve increases $lCMR_{glc}$ almost linearly with spike frequency in the superior cervical ganglion (4) or dorsal horn of the lumbar spinal cord (5), respectively (Fig. 2). The glucose consumed per single action potential can be determined from the slope of the straight line; it equals 0.4 nmol/g of tissue per spike in the superior cervical ganglion and 0.6 nmol/g per spike in the dorsal horn of the lumbar spinal cord (Fig. 2).

[1] Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD 20892-4030.

[2] Address reprint requests to: Dr. Louis Sokoloff, Laboratory of Cerebral Metabolism, National Institute of Mental Health, Building #36, 1A-05, 36 Convent Drive MSC 4030, Bethesda, MD 20892-4030. Telephone: 301-496-1371, FAX: 301-480-1668, Email: louis@shiloh.nimh.nih.gov.

*Special issue dedicated to Dr. Kunihiko Suzuki

Abbreviations Used: [^{14}C]DG, 2-deoxy-D-[1-^{14}C]glucose; $lCMR_{glc}$, local cerebral glucose utilization; $[K^+]_o$, extracellular K^+ concentration; $[Na^+]_i$, intracellular Na^+ concentration; dbcAMP, N-6,2'-O-dibutyryl cyclic AMP; THA, DL-threo-β-hydroxyaspartate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione.

0364-3190/99/0200-0321$16.00/0

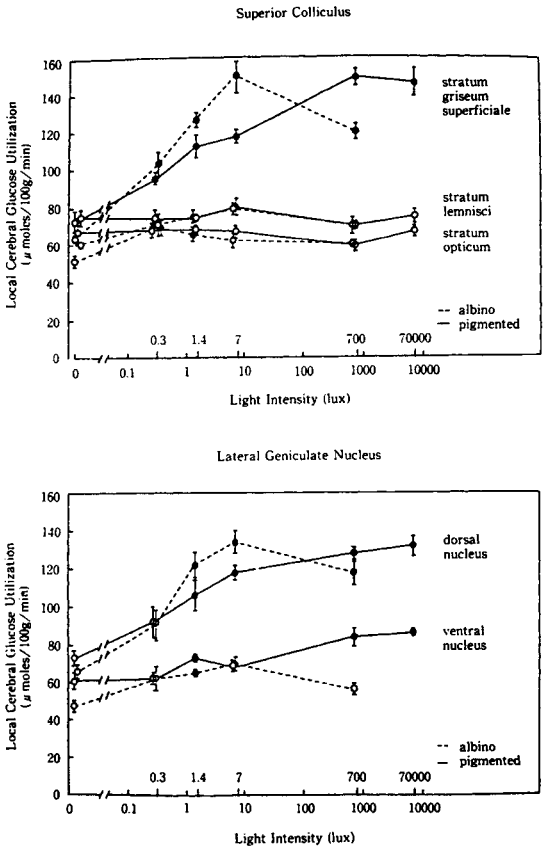

Fig. 1. Rates of glucose utilization as function of intensity of retinal illumination with randomly timed light flashes in various layers of superior colliculus and lateral geniculate nucleus of dark adapted albino and pigmented rats. From Miyaoka et al. (3).

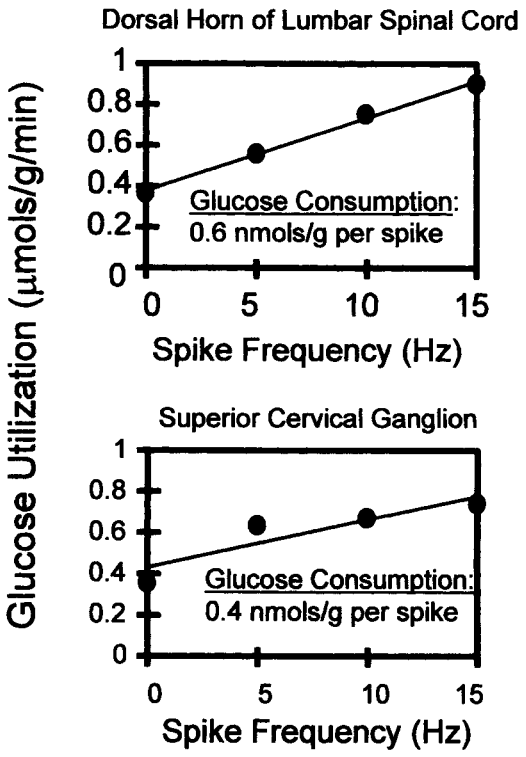

Fig. 2. Relationship between frequency of action potentials and glucose utilization in dorsal horn of lumbar spinal cord and in superior cervical ganglion during electrical stimulation of sciatic nerve or cervical sympathetic trunk at different frequencies, respectively. Derived from data of Kadekaro et al. (5) (Top) and Yarowsky et al. (4) (Bottom).

Biochemical Mechanisms of Functional Activation of Energy Metabolism at Tissue Level

Muscles move or support masses against gravitational forces; the heart pumps blood against a pressure head; and kidneys transport water and solutes against osmotic and concentration gradients. These functions all involve physical work that clearly require an expenditure of energy. It is less obvious what energy-requiring work nervous tissues do when they are functionally activated. Clearly, it must involve the generation, propagation, and conduction of action potentials inasmuch as the rate of glucose utilization increases almost linearly with spike frequency (Fig. 2). Action potentials are passive reflections of increased Na^+ influx and K^+ efflux in neurons and do not directly consume energy but draw on the potential energy stored in the membrane potentials. Therefore, with higher spike frequencies there should be greater increases in the concentrations of intracellular Na^+ ($[Na^+]_i$) and extracellular K^+ ($[K^+]_o$) and, therefore, the need for more Na^+,K^+-ATPase activity to restore the ionic gradients across the membranes to their resting levels. Increased ATPase activity would be expected to lower the ATP concentration and ATP/ADP ratio and raise the ADP, phosphate acceptor, and inorganic phosphate levels within the cell; these are all intracellular changes that can stimulate both glycolysis and electron transport and, therefore, energy metabolism. In many of the conditions in which the [^{14}C]DG method showed local increases in $lCMR_{glc}$, elevations in $[K^+]_o$ have been found in the affected tissues. To examine directly the effects of increased $[K^+]_o$ in vivo Shinohara et al. (6) applied K^+ directly to the cerebral cortex of rats, a procedure known to depolarize cell membranes and to cause spreading cortical depression, which itself raises $[K^+]_o$. Cerebral cortical glucose utilization was found to be profoundly increased as well (Fig. 3). The possibility that activation of Na^+,K^+-ATPase activity by membrane-depolarizing stimulation was responsible for the coupling of energy metabolism to functional activity was examined in rat neurohypophysis incubated in vitro under

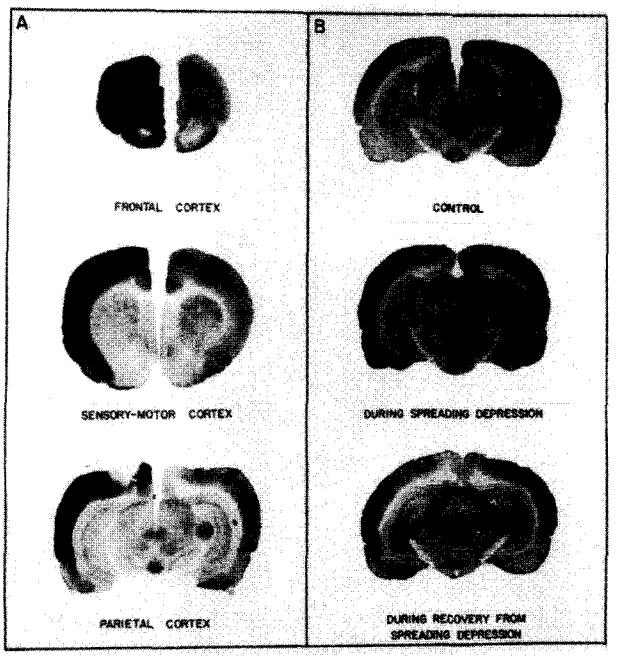

Fig. 3. [^{14}C]Deoxyglucose autoradiograms showing changes in $lCMR_{glc}$ during K^+-induced spreading cortical depression and during recovery in the rat; greater densities indicate higher $lCMR_{glc}$. In all cases the experimental side on the left was treated with KCl, and the control side on the right was treated with equivalent concentrations of NaCl. (A) Autoradiograms of brain sections at various levels of cerebral cortex from conscious rat in which spreading cortical depression was produced and sustained on left side by 5 M KCl applied to surface of intact dura over parietal cortex every 15–20 min; right side treated comparably with NaCl.
(B) Autoradiograms of brain sections at level of parietal cortex from three rats under barbiturate anesthesia. Top section is from normal, anesthetized rat; middle section is from similarly anesthetized rat in which 80 mM KCl in artificial cerebrospinal fluid was repeatedly applied directly to the surface of the left parietal cortex; bottom section is from similarly anesthetized rat studied immediately after return of cortical DC potential to normal after a single wave of spreading depression induced by a single application of 80 mM KCl to the parieto-occipital cortex of the left side. From Shinohara et al. (6).

conditions in which such stimulation promoted vasopressin secretion (7). Electrical stimulation raised [^{14}C]DG phosphorylation, indicating increased glucose utilization, and this increase was completely blocked by addition of ouabain, a specific inhibitor of Na^+,K^+-ATPase that does not inhibit spike generation or hormone secretion (Table IA). The cell membranes were also depolarized by addition of veratridine, which opens voltage-dependent Na^+ channels and allows Na^+ entry into the cells. Veratridine also markedly stimulated [^{14}C]DG phosphorylation, and again the stimulation was prevented by either ouabain or tetrodotoxin, the latter a blocker of voltage-gated Na^+ channels (Table IB). The increased [^{14}C]DG phosphorylation was not related to stimulation of vasopressin secretion; the posterior pituitary glands cannot be stimulated to secrete hormone in Ca^{++}-free medium, but even in such medium veratridine stimulated [^{14}C]DG phosphorylation (Table IC).

It appears then that the extra energy metabolism associated with electrical and functional activation in neural tissue is not used directly in the generation and propagation of action potentials. The energy is used rather to restore the ionic gradients and resting membrane potentials that were partly degraded by the action potentials. The functional activation of energy metabolism is, therefore, the source of what used to be called "the heat of recovery."

Localization of Functionally Activated Energy Metabolism at Tissue Level

Traditional electrophysiology focuses largely on spike activity in neuronal cell bodies. We initially assumed, therefore, that the increases in lCMRglc associated with functional activation were primarily in perikarya. Comparisons between the [^{14}C]DG autoradiograms and the stained brain sections from which they were prepared did not, however, support this assumption. In autoradiograms of normal rat brain the darkest (i.e., metabolically most active) region in the hippocampus was first thought to represent the pyramidal cell layer but was found to be the molecular layer, a synapse-rich, cell-poor region (Fig. 4). In the striate cortex of the normal monkey $lCMR_{glc}$ is highest in a sub-layer of Layer IV that is not particularly rich in cellular elements; it is the layer where axonal terminals of the afferent geniculocalcarine pathway synapse with dendrites of neurons situated in other laminae of the visual cortex (Fig. 5A, C) (8). It is in this neuropil-rich layer that $lCMR_{glc}$ is most reduced when visual input is interrupted (Fig. 5B). Osmotic stimulation by salt-loading markedly increases $lCMR_{glc}$ in the neurohypophysis 40% of which consists of axonal terminals derived from the afferent hypothalamo-neurohypophysial tract (9), while the supraoptic and paraventricular nuclei in the hypothalamus, the sites of the cell bodies where this tract originates, are essentially unaffected (Fig. 6) (10). On the other hand, hypotension produced by α-adrenergic blockade, hemorrhage, etc. markedly stimulates $lCMR_{glc}$ in these nuclei (Fig. 6). The difference is that osmotic stimulation acts directly on the cell bodies whereas hypotension exerts its effects on the cell bodies in these nuclei via afferent inputs from the brain stem (e.g., the nucleus tractus solitarius) that are part of the neural pathways of the baroreceptor reflexes. These results indicate that it is mainly the energy metabolism in regions rich in neuropil

Table I. Influence of Sodium Pump Activity and Neurosecretion on [^{14}C]Deoxyglucose Uptake in Posterior Pituitary In Vitro[a]

Condition	[^{14}C]Deoxyglucose Uptake (Cpm/100 μg protein/15 min)
A. Dependence on activation of sodium pump — Electrical Stimulation	
Controls (4)	988 ± 19
+ Electrical stimulation at 10 Hz (4)	1272 ± 57[b]
+ Electrical stimulation at 10 Hz + ouabain (4)	1018 ± 51[c]
B. Dependence on activation of sodium pump — Opening Na+ channels	
Controls (14)	1381 ± 50
+ Veratridine (14)	1891 ± 85[b]
+ Tetrodotoxin (9)	1209 ± 84[c]
+ Veratridine + Tetrodotoxin (8)	1551 ± 72[c]
+ Ouabain (4)	1318 ± 57[c]
+ Veratridine + Ouabain (4)	1218 ± 120[c]
C. Independence from activation of secretion (Ca^{2+} − free medium)	
Controls (in Ca^{2+} — free medium) (6)	1142 ± 38
+ Veratridine (in Ca^{2+} — free medium) (6)	1681 ± 78[b]

[a]The values represent means ± SEM of results obtained in number of experiments indicated in parentheses.
[b]Indicates statistically significant difference from controls ($p < 0.001$).
[c]Indicates no statistical significant difference from controls.
From Mata et al. (7)

and synapses and not cell bodies that is linked to functional activity.

In order to compare directly the effects of functional activation on $lCMR_{glc}$ in perikarya and nerve terminals in the same pathway, Kadekaro et al. (5) electrically stimulated the sciatic nerve of the anesthetized rat at different frequencies and measured $lCMR_{glc}$ in both the dorsal root ganglia and dorsal horn of corresponding segments of the lumbar spinal cord. The advantage of this system is that the body of the dorsal root ganglion contains perikarya devoid of neural processes, and cell bodies free of nerve terminals can, therefore, be examined simultaneously with the nerve terminals of the same pathway in the dorsal horn of the spinal cord. The results confirmed that it is the $lCMR_{glc}$ in the region of the nerve terminals and not the cell bodies that is linked to the functional activity. Glucose utilization in the dorsal horn of the lumbar spinal cord increased linearly with increasing frequency of stimulation but showed no changes in response to electrical stimulation in the region of the cell bodies of the same pathway in the dorsal root ganglia (Fig. 7). The failure of the perikarya in the dorsal root ganglion to respond metabolically to electrical stimulation may be surprising, but there is evidence that the soma membrane of neurons is not very excitable and not very productive of action potentials because of a relative paucity of voltage-gated Na^+ channels. Smith (11), using patch clamp electrodes, obtained evidence that soma and dendrites of spinal cord neurons and soma of dorsal root ganglion cells cultured in vitro do not generate action potentials. Freygang (12) and Freygang and Frank (13) had previously concluded from analyses

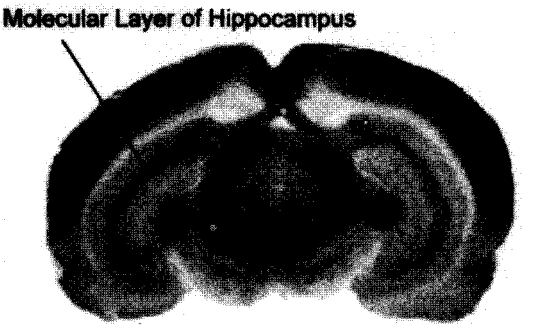

Fig. 4. [^{14}C]Deoxyglucose autoradiogram of normal, conscious rat brain. Greater optical density reflects greater $ICMR_{glc}$. Note high rate in molecular layer of hippocampus.

of extracellular potentials recorded from single spinal motor neurons and single neurons in the lateral geniculate nucleus that the soma-dendritic membrane can be driven synaptically to produce post-synaptic potentials but not propagating action potentials. If action potentials normally mediate the coupling of energy metabolism to functional activity and they are absent in the perikarya, then increased glucose metabolism due to functional activation is not to be expected. Some energy metabolism does, of course, proceed in cell bodies even at rest, but it is probably used more for vegetative and biosynthetic processes needed to maintain cellular structural and functional integrity rather than for processes directly related to functional activity.

The finding that rates of energy metabolism are linked to spike frequency in neuropil and not in cell

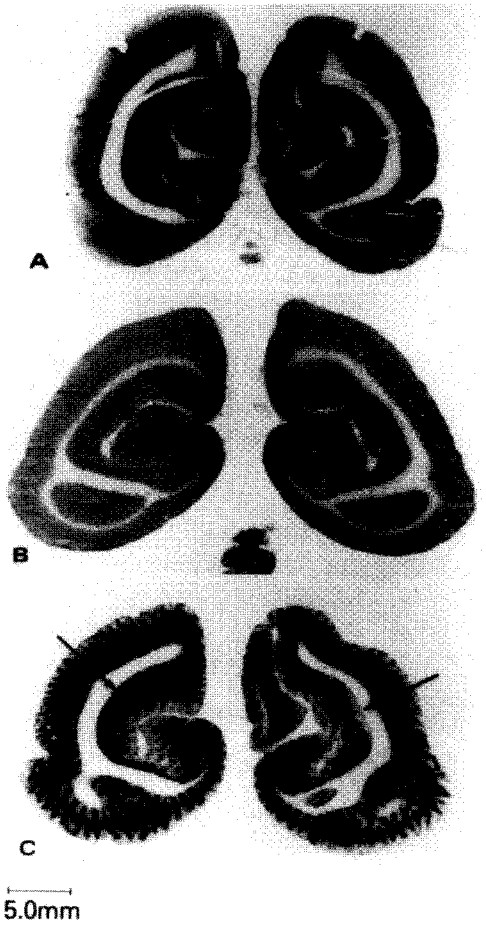

Fig. 5. [^{14}C]Deoxyglucose autoradiograms demonstrating effects of bilateral and monocular visual occlusion on $ICMR_{glc}$ in striate cortex of conscious monkey. Greater optical density indicates greater $ICMR_{glc}$. (A) Striate cortex from animal with both eyes open. Note heterogeneity in laminae; the darkest lamina corresponds to Layer IV. (B) Striate cortex from monkey with both eyes patched. Note general reduction in density and almost complete disappearance of laminar heterogeneity, especially in Laye IV. (C) Striate cortex from animal with only right eye patched. Left half of autoradiogram corresponds to left hemisphere contralateral to occluded eye. Note alternating dark and light columns traversing the full thickness of the striate cortex which represent the ocular dominance columns. The dark bands represent the columns for the open eye; the light bands represent the columns for the patched eye and demonstrate the reduced glucose utilization resulting from reduced visual input. The arrows point to regions of bilateral asymmetry; these are the loci of representation of the blind spots of the visual fields. From Kennedy et al. (8).

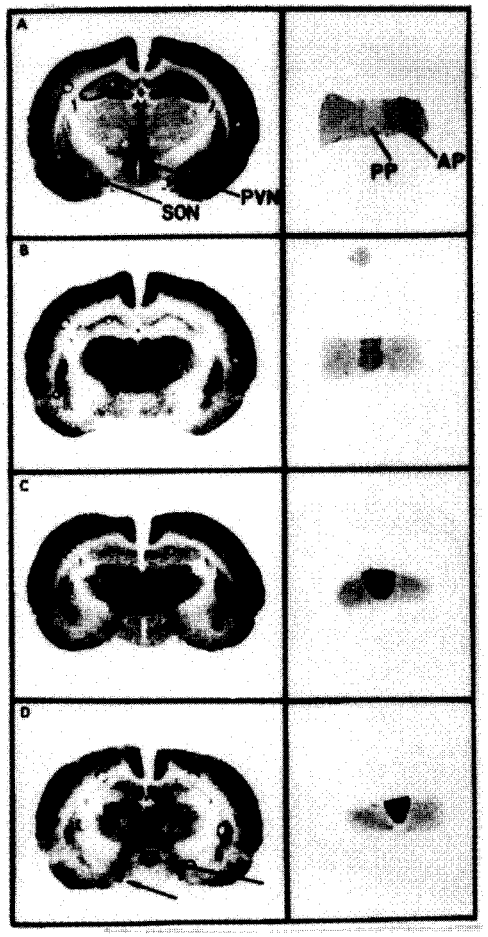

Fig. 6. Effects of activation of hypothalamo-neurohypophysial pathway by salt-loading or hypotension on $ICMR_{glc}$ in the conscious rat. (A) Histological sections of brain stained with cresyl violet (Nissl) and pituitary stained with toluidine blue demonstrating positions of supraoptic nucleus (SON), paraventricular nucleus (PVN), posterior pituitary (PP), and anterior pituitary (AP). (B) [^{14}C]Deoxyglucose autoradiograms of brain and pituitary from normal control rat drinking only water. (C) [^{14}C]Deoxyglucose autoradiograms from rat given 2% (w/v) NaCl in drinking water for 5 days. Note selective marked increase in density in posterior hypophysis, indicating increased glucose utilization. (D) [^{14}C]Deoxyglucose autoradiograms from rat made hypotensive by administration of 20 mg/kg of phenoxybenzamine 45–60 minutes prior to administration of the [^{14}C]deoxyglucose. Note selective increases in labeling of supraoptic and paraventricular nuclei and posterior pituitary. From Schwartz et al. (10).

bodies should help to resolve questions about possible differences in the metabolic responses to functional excitation and inhibition. Increases in glucose utilization have been observed in structures in which electrophysiological evidence indicated inhibition of neuronal activity, thus raising questions whether active inhibition required energy just like excitation. It appears, however, that it is the spike activity in the afferent nerve terminals that is responsible for the energy consumption, and this activity is the same whether the terminals are releasing excitatory or inhibitory neurotransmitters. The energy metabolism of the post-synaptic cell bodies, whether activated or inhibited, is not significantly altered, and to determine which has occurred, it is necessary to look downstream at the next synapses in the projection zones

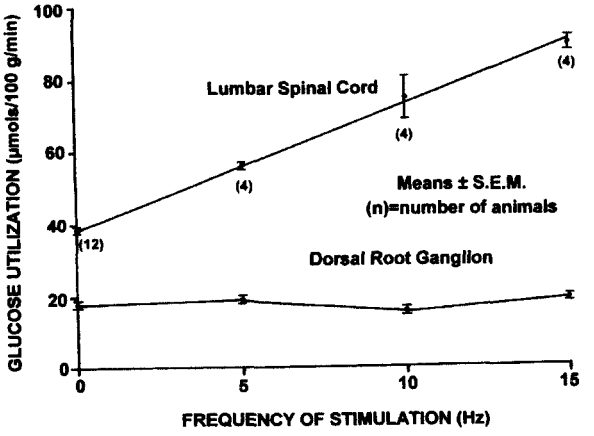

Fig. 7. Frequency-dependent effects of electrical stimulation of sciatic nerve on $ICMR_{glc}$ in dorsal root ganglion and dorsal horn of lumbar spinal cord. Error bars represent SEM. From Kadekaro et al. (5).

of those neurons. Glucose utilization is depressed in the projection zones of inhibited neurons and increased in the projection zones of excited neurons.

Cellular Localization of Functional Activation of Energy Metabolism

The neuropil, in which functionally activated energy metabolism is localized, contains not only neuronal elements, e.g., axonal terminals, dendritic processes, and synapses, but also astrocytic processes as well. Because increases in $1CMR_{glc}$ are directly related to frequency of spike activity and linked to Na^+, K^+-ATPase activity, and action potentials are reflections of increased Na^+ influx and K^+ efflux in neurons, it is likely that some of the extra energy metabolism is used to restore the ionic gradients in the neuronal elements where the action potentials are produced. Astrocytic processes, which envelop the synapses, might also be involved in the metabolic response. Astrocytes are believed to regulate $[K^+]_o$ either by passive diffusion (14,15) and/or active transport (16) following increases in $[K^+]_o$ resulting from neuronal excitation (17,18), and studies with tissue slices (19,20) or cultured cells (21–25) have indicated that energy is consumed in the process. The spatial resolution of the autoradiographic deoxyglucose method is limited to 100–200 µm (26) which is inadequate to identify the specific cellular or subcellular elements in neuropil that contribute to the increases in $1CMR_{glc}$ during functional activation. Therefore, to approach this question, changes in the extracellular medium expected to result from increased spike activity in vivo were simulated in vitro, and their effects on glucose metabolism of cultured neurons and astroglia were studied. Spike activity in neuronal processes leads to increased extracellular K^+ and intracellular Na^+ concentrations and release of neurotransmitters. Therefore, the effects of elevated $[K^+]_o$ and $[Na^+]_i$, and of glutamate, the most prevalent excitatory neurotransmitter in brain, on glucose utilization in cultured neurons and astroglia were examined (27). Neuronal and mixed neuronal-astroglial cultures were prepared from striatum of fetal rats (embryonic day 16) and used in assays after 6–8 days in culture. Astroglial cultures were prepared from cerebral cortex of newborn rats and assayed when confluent after 19–29 days in culture. Glucose utilization was assayed by measurement of the [^{14}C]deoxyglucose-6-phosphate formed and accumulated in the cells during incubation at 37°C.

Effects of Increased $[K^+]_o$. Increased $[K^+_o]$ in the range of 5.4 to 56 mM during the assay significantly stimulated [^{14}C]DG phosphorylation in both neuronal and mixed neuronal-astroglial cultures after incubations of 15 and 30 minutes (Fig. 8) (27). This effect in neurons was found to be ouabain-sensitive, indicating involvement of Na^+,K^+-ATPase in the mechanism of the effect (data not shown). In contrast, raising $[K^+]_o$ in the same range had no such effect in the cultured astroglia. In fact, the highest levels of $[K^+]_o$ examined tended to inhibit [^{14}C]DG phosphorylation in astroglia (Fig. 8). Addition of dibutyryl cAMP (dbcAMP) to the culture medium transform astroglia from protoplasmic to fibrous type; this raises baseline rates of [^{14}C]DG phosphorylation but does not result in stimulation of [^{14}C]DG phosphorylation by elevated $[K^+]_o$ (27).

Effects of Increased $[Na^+]_i$. Contrary to the lack of effect by increased $[K^+]_o$, Na^+ entry into the cells promoted either by veratridine, which opens membrane voltage-gated Na^+ channels, or by monensin, a Na^+ ionophore, markedly stimulates [^{14}C]DG phosphorylation in astroglia, provided Na^+ is present (Fig. 9) (23,27). Tetrodotoxin, which blocks voltage-dependent Na^+ channels, does not alter baseline rates of [^{14}C]DG phosphorylation in astroglia; it does, however, prevent stimulation by veratridine but has no effect on the monensin stimulation (Fig. 9). Ouabain, the Na^+,K^+-ATPase inhibitor, lowers basal rates of [^{14}C]DG phosphorylation and completely blocks the stimulation by veratridine, but only partially suppresses the monensin stimulation (Fig. 9). The absence of a tetrodotoxin effect and an only partial ouabain effect on the monensin stimulation can be explained by the fact that monensin-induced Na^+ entry is not through voltage-dependent Na^+ channels and is, therefore, insensitive to blockade by tetrodotoxin. Monensin is a Na^+/H^+ exchanger that exchanges extracellular Na^+ for intracellular H^+, raising Na^+ and lower-

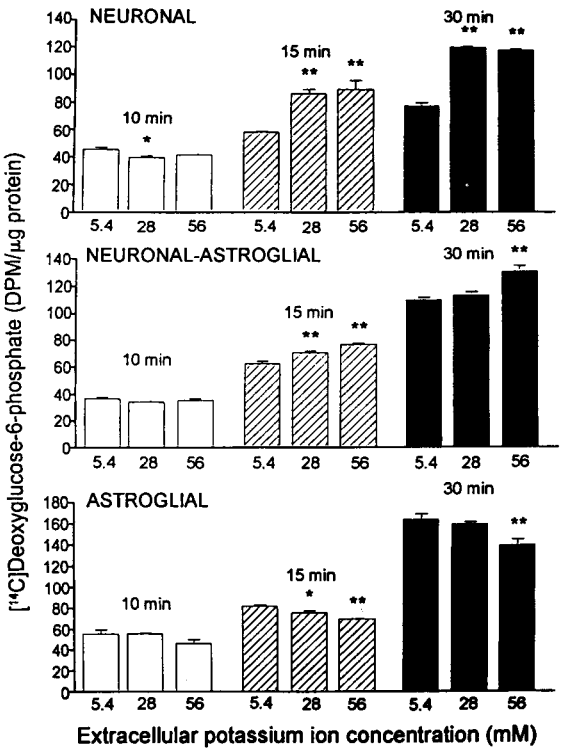

Fig. 8. Effects of $[K^+]_o$ on rates of [^{14}C]DG phosphorylation in cultured neurons (6-day old culture), mixed neuronal-astroglia (6-day old culture), and astroglia (19-day old culture, no dbcAMP). Values are means ± SEM obtained from quadruplicate wells. Numbers above bars indicate duration of incubation. *$p < 0.05$, **$p < 0.01$ compared to the 5.4 mM $[K^+]_o$ (Dunnett's test for multiple comparisons). Representative of at least three such experiments for each condition. From Takahashi et al. (27).

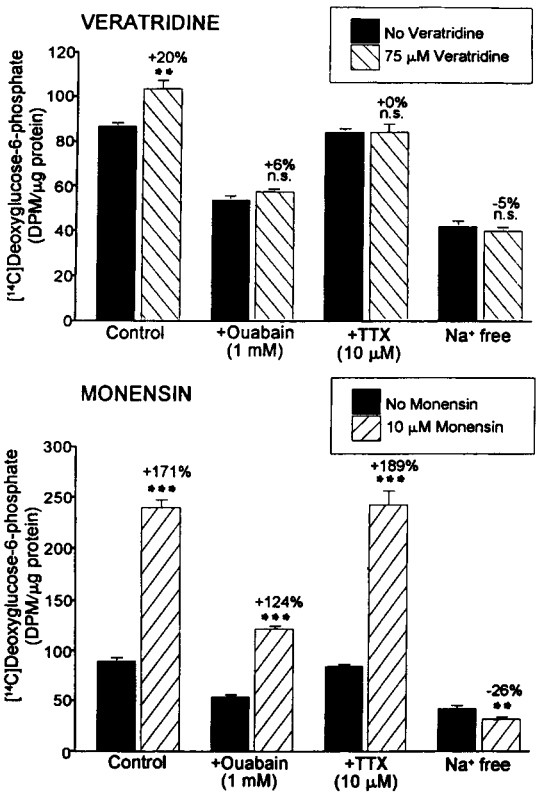

Fig. 9. Effects of veratridine (upper) and monensin (lower) on [^{14}C]DG phosphorylation in astroglia (21-day old culture, no dbcAMP) and the effects of ouabain, tetrodotoxin (TTX), and Na^+-free medium on the veratridine and monensin effects. Values are means ± SEM obtained from quadruplicate wells. Numbers above bars indicate percent difference from each control. Representative of three such experiments in three different astroglial preparations. From Takahashi et al. (27). **$p < 0.01$; ***$p < 0.001$; n.s., not statistically significant from each control (grouped t test).

ing H^+ concentrations in the cells. Both ionic changes can enhance glucose metabolism, the former by ouabain-sensitive stimulation of Na^+,K^+-ATPase activity, and the latter by ouabain-insensitive stimulation of phosphofructokinase activity due to increased intracellular pH.

Effects of Glutamate on [^{14}C]Deoxyglucose Phosphorylation in Astroglia. In contrast to the lack of an effect of elevated $[K^+]_o$, glutamate stimulates [^{14}C]DG phosphorylation in astroglia (27,28) (Fig. 10). This stimulation is Na^+-dependent, ouabain-sensitive, unaffected by tetrodotoxin, and insensitive to NMDA and non-NMDA glutamate receptor antagonists (Fig. 10). DL-Threo-β-hydroxyaspartate, a competitive inhibitor of glutamate reuptake by the Na^+/glutamate co-transport system (29), itself stimulates [^{14}C]DG phosphorylation, and glutamate no longer stimulates in its presence. The hydroxyaspartate stimulation is also blocked by ouabain, absent in Na^+-free medium, and unaffected by tetrodotoxin and inhibitors of NMDA or non-NMDA receptors. These results indicate that glutamate does stimulate glucose utilization in astroglia but not by actions at glutamate receptors or voltage-dependent Na^+ channels. It is instead mediated by increased $[Na^+]_i$ resulting from co-transport of Na^+ with glutamate reuptake by the Na^+/glutamate co-transporter.

CONCLUSIONS

The main work of neural tissues that requires energy derived from metabolism is clearly related to the generation and propagation of action potentials which carry the information neurons transmit to one another. Normally, the almost sole substrate for energy metabolism in neural tissues is glucose, and its utilization increases almost linearly with spike frequency in functionally activated regions. Spikes reflect Na^+ efflux

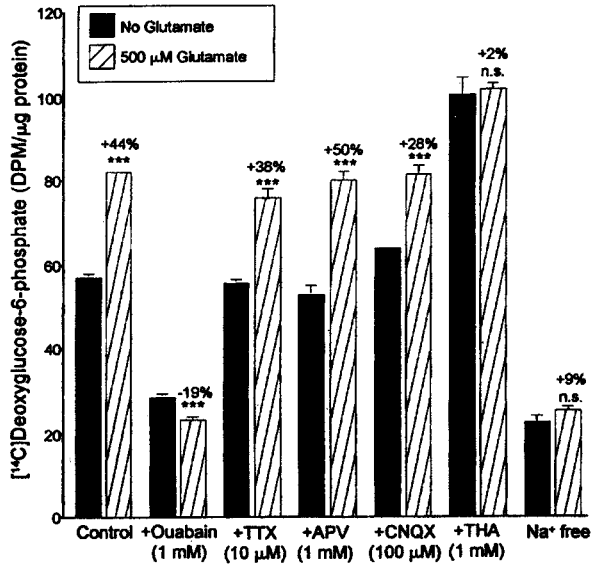

Fig. 10. Effects of glutamate on [^{14}C]DG phosphorylation in astroglia (22-day old cultures, no dbcAMP) and the effects of DL-2-amino-5-phosphonovaleric acid (APV), CNQX, ouabain, tetrodotoxin (TTX), DL-threo-β-hydroxyaspartic acid (THA), and Na^+-free medium on the glutamate effects. Values are means ± SEM obtained from quadruplicate wells. Numbers above bars indicate peRcent differences from each control. Representative of three such experiments with three different astroglial preparations.
***$p < 0.001$; n.s., not statistically significant, from each control group (grouped t test). From Takahashi et al. (27).

and K^+ influx in the neuronal elements where they are generated, and the greater the spike frequency the greater the ionic displacements. The increased extracellular K^+ and intracellular Na^+ concentrations, particularly the latter, stimulate Na^+,K^+-ATPase activity to restore the ionic gradients to their normal resting levels, and energy metabolism is then increased to resynthesize the ATP consumed in that process. Functional activation of glucose utilization is essentially confined to neuropil which contains axonal, dendritic, and astrocytic processes. Obviously, some of the increased energy metabolism must occur in the axonal terminals and dendrites where the spikes are generated. Functional activation of energy metabolism is not seen in perikarya, presumably because they have few voltage-dependent Na^+ channels and, therefore, generate few if any action potentials.

Astrocytes contribute to the increased energy metabolism in neuropil during functional activation, but by other mechanisms. Astrocytic membranes can be depolarized by increased $[K^+]_i$, but they do not produce action potentials that would allow the Na^+ influx into the cells needed to stimulate Na^+,K^+-ATPase activity. Action potentials in axonal terminals, however, are associated with release of neurotransmitters. Glutamate is the most prevalent excitatory neurotransmitter in brain, but it is also a neurotoxin, and its extracellular concentration must be kept low by astrocytic reuptake via Na^+-dependent glutamate transporters that transport 2–3 Na^+ ions with each glutamate molecule into the cell. The increased $[Na^+]_i$ then results in stimulation of Na^+,K^+-ATPase activity and energy metabolism.

Astrocytic processes surround the capillaries that bring glucose to the brain, and some if not all the glucose must traverse them before reaching the neurons. Evidence has been accumulating that astrocytes first metabolize glucose to lactate which they then release to the neurons for oxidation to CO_2 and H_2O (30,31). If so, then increased glucose utilization by astrocytes may be essential for the increased energy metabolism by neuronal elements during functional activation.

REFERENCES

1. Sokoloff. L., Reivich, M., Kennedy, C., Des Rosiers, M. H., Patlak, C. S., Pettigrew, K. D., Sakurada, O., and Shinohara, M. 1977. The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem. 28: 897–916.
2. Sokoloff, L. 1981. Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. J. Cereb. Blood Flow Metab. 1:7–36.
3. Miyaoka, M., Shinohara, M., Batipps, M., Pettigrew, K. D., Kennedy, C., and Sokoloff, L. 1979. The relationship between the intensity of the stimulus and the metabolic response in the visual system of the rat. Acta. Neurol. Scand. 60 (Suppl. 70):16–17.
4. Yarowsky, P., Kadekaro, M., and Sokoloff, L. 1983. Frequency-dependent activation of glucose utilization in the superior cervical ganglion by electrical stimulation of cervical sympathetic trunk. Proc. Natl. Acad. Sci., USA 80:4179–4183.
5. Kadekaro, M., Crane, A. M., and Sokoloff, L. 1985. Differential effects of electrical stimulation of sciatic nerve on metabolic activity in spinal cord and dorsal root ganglion in the rat. Proc. Natl. Acad. Sci., USA 82:6010–6013.
6. Shinohara, M., Dollinger, B., Brown, G., Rapoport, S., and Sokoloff, L. 1979. Cerebral glucose utilization: Local changes during and after recovery from spreading cortical depression. Science 203:188–190
7. Mata, M., Fink, D. J., Gainer, H., Smith, C. B., Davidsen, L., Savaki, H., Schwartz, W. J., and Sokoloff, L. 1980. Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity. J. Neurochem. 34:213–215.
8. Kennedy, C., Des Rosiers, M. H., Sakurada, O., Shinohara, M., Reivich, M., Jehle, J. W., and Sokoloff, L. 1976. Metabolic mapping of the primary visual system of the monkey by means of the autoradiographic [^{14}C]deoxyglucose technique. Proc. Natl. Acad. Sci., USA 73:4230–4234.
9. Nordmann, J. J. 1977. Ultrastructural morphometry of the rat neurohypophysis. J. Anat. 123:213–218.
10. Schwartz, W. J., Smith, C. B., Davidsen, L., Savaki, H., Sokoloff, L., Mata, M., Fink, D. J., and Gainer, H. 1979. Metabolic mapping of functional activity in the hypothalomo-neurohypophysial system of the rat. Science 205:723–725.
11. Smith, T. G, Jr. 1983. Sites of action potential generation in cultured neurons. Brain Res. 288:381–383.

12. Freygang, W. H. Jr. 1958. An analysis of extracellular potentials from single neurons in the lateral geniculate nucleus of the cat. J. Gen. Physiol. 41:543–564.
13. Freygang, W. H., Jr. and Frank, K. 1959. Extracellular potentials from single spinal motoneurones. J. Gen. Physiol. 42:749–760.
14. Orkand, R. K., Nicholls, J. G, and Kuffler, S. W. 1966. Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. J. Neurophysiol. 29:788–806.
15. Medzihradsky, F, Nandhasri, P. S., Idoyaga-Vargas, V, and Sellinger, O. Z. 1971. A comparison of ATPase activity of the glial cell fraction and the neuronal perikaryal fraction isolated in bulk from rat cerebral cortex. J. Neurochem. 18:1599–1603.
16. Henn, F. A., Haljamäe, H., and Hamberger, A. 1972. Glial cell function: active control of extracellular K^+ concentration. Brain Res. 43:437–443.
17. Hertz, L. 1977. Drug-induced alterations of ion distribution at the cellular level of the central nervous system. Pharmacol. Rev. 29: 35–65.
18. Erecinska, M. and Silver, I. A. 1994. Metabolism and role of glutamate in mammalian brain. Progress in Neurobiol. 43:37–71.
19. Yarowsky, P., Boyne, A. F., Wierwille, R., and Brookes, N. 1986. Effect of monensin on deoxyglucose uptake in cultured astrocytes: energy metabolism is coupled to sodium entry. J. Neurosci. 6:859–866.
20. Badar-Goffer, R. S., Ben-Yoseph, O., Bachelard, H. S., and Morris, P. G. 1992. Neuronal-glial metabolism under depolarizing conditions. A ^{13}C-n.m.r. study. Biochem. J. 282:225–230.
21. Cummins, C. J., Glover, R. A., Sellinger, O. Z. 1979a. Neuronal cues regulate uptake in cultured astrocytes. Brain Res. 170:190–193.
22. Cummins, C. J., Glover, R. A., Sellinger, O. Z. 1979b. Astroglial uptake is modulated by extracellular K^+. J. Neurochem. 33:779–785.
23. Brookes, N., Yarowsky, P. J. 1985. Determinants of deoxyglucose uptake in cultured astrocytes: the role of the sodium pump. J. Neurochem. 44:473–479.
24. Hertz, L., and Peng, L. 1992. Energy metabolism at the cellular level of the CNS. Can. J. Physiol. Pharmacol. 70 (Suppl.):S145–S157.
25. Peng, L., Zhang, X., and Hertz, L. 1994. High extracellular potassium concentrations stimulate oxidative metabolism in a glutamatergic neuronal culture and glycolysis in cultured astrocytes but have no stimulatory effect in a GABAergic neuronal culture. Brain Res. 663:168–172.
26. Smith, C. B. 1983. Localization of activity-associated changes in metabolism of the central nervous system with the deoxyglucose method: Prospects for cellular resolution. Pages 269–317, in Barker, J. L., and McKelvy, J. F. (eds.), Current Methods in Cellular Neurobiology, Vol. I, Anatomical Techniques, John Wiley, New York.
27. Takahashi, S., Driscoll, B. F., Law, M. J., and Sokoloff, L. 1995. Role of sodium and potassium in regulation of glucose metabolism in cultured astroglia. Proc. Natl. Acad. Sci. USA 92:4616–4620.
28. Pellerin, L., and Magistretti, P. J. 1994. Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. Proc. Natl. Acad. Sci. USA 91:10625–10629
29. Flott, B., and Seifert, W. 1991. Characterization of glutamate uptake in astrocyte primary cultures from rat brain. Glia 4:293–304
30. Magistretti, P. J., and Pellerin, L. 1996. Cellular bases of brain energy metabolism and their relevance to functional brain imaging: Evidence for a prominent role of astrocytes. Cerebral Cortex 6:50–61.
31. Tsacopoulos, M., and Magistretti, P. 1996. Metabolic coupling between glia and neurons. J. Neurosci. 16:877–885.